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(54) Title: SEQUENCE

(57) Abstract: The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against infection by *Piscirickettsia salmonis*. The invention is based on or derived from the nucleic acid or amino acid sequence of at least a part of the surface antigen present on *Piscirickettsia salmonis*. Nucleic acid and/or amino acid sequences may be used in the preparation of a vaccine to protect against infection by *Piscirickettsia salmonis*.

"Sequence"

The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against piscirickettsiosis also referred to as salmonid rickettsial septicaemia (SRS).

To date no commercially available vaccine is effective against *Piscirickettsia salmonis*, the causative agent of SRS. Accordingly there is a need for an effective vaccine against *Piscirickettsia salmonis*.

It is an object of the present invention to provide a vaccine to protect against SRS.

It is an object of the present invention to provide a vaccine to protect against *Piscirickettsia salmonis*.

clone3/3PST-R, clone3.3APA-F, clone7/original, clone7/XbaR, clone7/7MunR, clone7/7MunF, clone20/original, clone20/20VSPF or clone 15.

Typically the amino acid sequence is derived from one of the above-mentioned nucleic acid sequences or is chosen from the sequences of p45 or HSP70 antigen.

Preferably peptide sequences or nucleic acid sequence identified herein as sequence ID numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 are utilised in a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

The invention provides the use of nucleic acid sequences or peptide sequence as defined herein in the preparation of a vaccine for the protection of fish against *Piscirickettsia salmonis*.

The invention further provides a vaccine to protect fish against *Piscirickettsia salmonis* wherein the vaccine includes nucleic acid or peptide sequences as defined herein.

Sequence ID 1, 2 and 3

A detailed description of the invention, listing the exact nucleotide sequence of Psclone51A and the

The present invention relates to a fish vaccine. More specifically the invention relates to a nucleic acid vaccine for administration to fish to protect against piscirickettsiosis also referred to as salmonid rickettsial septicaemia (SRS).

The invention further provides an amino acid sequence for administration to fish to protect agsint SRS.

In a further aspect the present invention provides at least one nucleic acid sequence and / or an amino acid sequence which is derived from *Piscirickettsia salmonis* or a synthetically prepared analogue thereof or a substantially homologous sequence.

A substantially homologous nucleic acid sequence is a sequence which can be transcribed and/or translated to provide an amino acid sequence which is substantially homologous to at least a part of a surface antigen present on *Piscirickettsia salmonis*.

A substantially homologous amino acid sequence encodes at least 70% of a part of a surface antigen and induces an immune response.

More preferably the homologous amino acid sequence will encode at least 90% of the amino acid sequence.

A typical nucleic acid sequence is chosen from the sequences of Psclone51A p10.6 (also known as immuno-reactive clone 0110-2-5), IcmE, clone3/original,

impact on the reduction of mortality of farmed Atlantic salmon caused by *Piscirickettsia salmonis*. Characterisation of the gene product will lead to the identification of key elements in pathogenesis of infection and to the design of more accurate diagnostic tests which will also aid in epidemiological studies documenting the dissemination of different strains of the disease.

The nucleotide sequence Psclone51A and associated derivatives thereof when translated into protein sequence being composed of either identical or equivalent amino acids, should induce a response by the hosts' immune system. This principle can be further expanded to use these proteins in diagnostics tests and vaccination procedures.

Sequence ID No 4 and 5

A detailed description of the invention, listing the exact nucleotide sequence of p10.6 and the amino acid sequence deduced for the open reading frame (ORF), is provided in figure 4 and figure 5 respectively.

The genetic sequence of *Piscirickettsia salmonis* (Atcc strain VR-1361), grown in CHSE-214 cells using homologous anistera from *Piscirickettsia salmonis* immunised rabbits, an immuno-reactive clone (0110-2-5) (p10.6) was identified from the expression library. This clone was sequenced and polymerase chain reaction (PCR) primers developed.

amino acid sequence deduced for the open reading frame (ORF), is provided in figures 1, 2 and 3. The genetic sequence has been derived from cloned cDNA wherein the cDNA clones were derived from *Piscirickettsia salmonis* type strain (LF-89) messenger RNA (mRNA). The cloned material was sequenced in both directions from the 5' and 3' insertion sites using overlapping amplicons.

Veracity of the Psclone51A sequence was confirmed by Polymerase Chain Reaction(PCR) and Reverse-Transcriptase PCR (RT-PCR) of appropriate sized amplicons from *Piscirickettsia salmonis* infected Chinook salmon embryonic (CHSE-214) cell line and not from uninfected control material. Expression of the cloned sequence has yielded a protein of approximately 12 kDa.

The ORF of Psclone51A described in figure 1 does not have any significant homology at the nucleotide level with previous submissions to databases accessible by BLAST. At the protein level, a border line similarity with a hypothetical 21.5 kDa protein of *Escherichia coli* was found.

The ORF has commercial value for the following reasons:

There is sufficient reason to believe that the nucleotide corresponding amino acid sequence is of *Piscirickettsia salmonis* origin. As such its incorporation into nucleic acid vaccines may have an

The *Piscirickettsia salmonis* origin of the p10.6 clone was confirmed through PCR amplification of *Piscirickettsia salmonis* and Chinook salmon embryonic cell line (CHSE-214) DNA using clone specific primers. Appropriately sized amplicons were amplified from the *Piscirickettsia salmonis* DNA, but not from the CHSE-214 DNA confirming that the immuno-reactive clone was of *Piscirickettsia salmonis* origin and not from the host cell DNA.

The open reading frame (ORF) for the full gene was completed by inverse PCR from genomic *Piscirickettsia salmonis* DNA.

The ORF of p10.6 described in figure 4 does not have any significant homology at the nucleotide level with previous submissions to databases accessible by BLAST. The derived amino acid sequence, but not the nucleotide sequence, shows significant homology to the 17kDa antigen found in *Rickettsia* of the Spotted Fever Group, where it is considered a group specific, outer membrane protein.

Sequence ID No 6

A typical nucleic acid sequence is IcmE (403).

Preferably peptide sequences transcribed or translated from the nucleic acid sequences of IcmE (403) are incorporated into a vaccination strategy

to induce an immune response to a surface antigen of *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of IcmE (403) and the amino acid sequence deduced for the open reading from (OFR), is provided in figure 6.

The genetic sequence has been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* type strian (LF-89) genomic DNA (gDNA). The IPCR product was sequenced in both direction from the 5' and 3' sides using overlapping amplicons.

The protein encoded by the ORF of IcmE (403) has a 37% significant homology at the protein level to the IcmE protein of *Legionella pneumophila* when compared to previous submissions to databases accessible by BLAST.

Sequence ID No 7

A typical amino acid sequenes is p45.

Preferably a nucleic acid sequence transcribing the amino acid sequence of p45 are incorporated into a vaccination strategy to induce an immune response to a surface antigen against *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact amino acid sequence of a portion of the p45 major antigen, is provided in figure 7.
The amino acid sequence has derived from microsequencing of a protein approximately 45 kDa found to be immunoreactive to rabbit anti-P salmonis antibodies. Moreover, p45 was found uniquely in Chinook salmon embryonic (SHSE-214) cells infected with *Piscirickettsia salmonis* and not in infected CHSE-214 cells.

The amino acid sequence of p45 has no significant homology to other bacterial proteins when compared to previous submissions to databases accessible by BLAST.

Sequence ID No 8, 9 and 10

A typical nucleic acid sequence is clone3/original, clone3/3PST-R, and clone3.3APA-F.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone3/original, clone3/3PST-R, and clone3.3APA-F are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* genomic DNA (gDNA).

The proteins encoded by the ORF of clone3/original, clone3/3PST-R and clone3.3APA-F have respectively 40%, 38% and 34% significant homology at the protein level to different portion of the transposase

protein of *Vibrio anguillarum* (gb AAA81776.1) when compared to previous submissions to databases accessible by BLAST.

Sequence ID No 11, 12, 13 and 14

A typical nucleic acid sequence is clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the extract nucleotide sequence of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF and the amino acid sequence deduced for their open reading frames (ORF), is provided in figures 11, 12, 13 and 14 respectively.

Some of the genetic sequences have been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* genomic DNA (gDNA).

The peptides encoded by the ORF of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF have a 40% to 44% significant homology at the protein level to different portion of the ABC transporter ATP-

binding protein of the other bacterial species when compared to previous submissions to databases accessible by BLAST.
There is sufficient reason to believe that the nucleotide and corresponding amino acid sequence are of *Piscirickettsia salmonis* origin. Also, part of the ORFs was found in an immuno-reactive clone of an expression library.

Sequence ID No 15 and 16

A typical nucleic acid sequence is clone20/original, and clone20/20VSPF.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone20/original, and clone20/20VSPF are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of clone20/original, and clone20/20VSPF and the amino acid sequence deduced for their open reading frames (ORF), is provided in figure 15 and figure 16 respectively.

Some of the genetic sequences have been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* genomic DNA (gDNA).

The peptides encoded by the ORF of clone20/original, and clone20/20VSPF have a 41% and 51% significant homology at the protein level to an amino acid transporter/permase protein of other organisms when compared to previous submissions to databases accessible by BLAST.

Sequence ID No 17

A typical nucleic acid sequence is clone15/original.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone15/original are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of clone15/original and the amino acid sequence deduced for its open reading frames (ORF), is provided in figure 17.

The nucleotide sequence and the peptide encoded by the ORF of clone15/original have no significant homology to proteins of other bacterial species when compared to previous submissions to databases accessible by BLAST.

From the previous information there is sufficient reason to believe that the nucleotide and

binding protein of the other bacterial species when compared to previous submissions to databases accessible by BLAST.
There is sufficient reason to believe that the nucleotide and corresponding amino acid sequence are of *Piscirickettsia salmonis* origin. Also, part of the ORFs was found in an immuno-reactive clone of an expression library.

Sequence ID No 15 and 16

A typical nucleic acid sequence is clone20/original, and clone20/20VSPF.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone20/original, and clone20/20VSPF are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of clone20/original, and clone20/20VSPF and the amino acid sequence deduced for their open reading frames (ORF), is provided in figure 15 and figure 16 respectively.

Some of the genetic sequences have been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* genomic DNA (gDNA).

immuno-reactive clones of an expression library. Together, this shows the potential of these ORFs to be incorporated into nucleic acid vaccines that may have an impact on the reduction of mortality of farmed salmonids due to *Rickettsial* Salmonid Septicaemia caused by *Piscirickettsia salmonis*.

PCR primers derived from the full ORF, used in touchdown PCR are capable of detecting *Piscirickettsia salmonis* in tissues from infected fish. This will lead to more accurate diagnostic tests to be used for clinical as well as epidemiological study purposes.

The nucleotide sequences associated derivatives thereof when translated into protein sequence being composed of either identical or equivalent amino acids, should induce a response by the hosts' immune system. This principle can be further expanded to use these proteins in diagnostic tests and nucleic acid vaccination procedures.

CLAIMS

1. A nucleic acid which can be transcribed to provide an amino acid sequence which is substantially homologous to at least a part of the surface antigen present on *Piscirickettsia salmonis*.

2. A nucleic acid sequence as claimed in claim 1 which encodes a part of a surface antigen of *Piscirickettsia salmonis* which induces an immune response.

3. A nucleic acid sequence as claimed in claim 1 or claim 2 wherein the nucleic acid sequence is herein chosen from the sequences described as sequence ID numbers 1, 2, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17.

4. Use of the nucleic acid sequences set out in any of the preceding claims in the preparation of a vaccine to protect against infection by *Piscirickettsia salmonis*.

5. An amino acid sequence derived from the sequence of any of claims 1 to 4.

6. Use of at least one amino acid sequence chosen from sequence ID numbers 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 in the preparation of a vaccine to protect fish against *Piscirickettsia salmonis*.

7. A nucleic acid based vaccine comprising at least one sequence chosen form the sequences described herein under sequence ID numbers 1, 2, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17.

8. An amino acid based vaccine against *Piscirickettsia salmonis* comprising at least one sequence chosen from the amino acid sequences described herein under ID numbers 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18.

			10									
tga	gtg	gcg	agg	aga	taa	gct	aaa	atg	agt	caa	gca	tta
								M	S	Q	A	L
			50									
gtt	gaa	ttt	aaa	acg	gta	gat	cat	gtg	ctg	caa	gcc	tta
V	E	F	K	T	V	D	H	V	L	Q	A	L
							100					
ggc	gtt	gaa	ggg	atg	agt	gca	gcg	gat	gtg	cat	ggc	ctg
G	V	E	G	M	S	A	A	D	V	H	G	L
										150		
ctg	att	ggt	atg	ttg	gcg	agt	caa	agt	aat	tta	acc	tgt
L	I	G	M	L	A	S	Q	S	N	L	T	C
aaa	tca	tgg	tta	gaa	aaa	gcg	ata	ttt	atg	gga	gct	cac
K	S	W	L	E	K	A	I	F	M	G	A	H
	200											
ctt	gat	gct	gaa	agt	gat	ctt	ttt	agt	aat	atc	atg	gcc
L	D	A	E	S	D	L	F	S	N	I	M	A
					250							
aaa	gag	cag	tta	aag	cag	tta	gag	gca	ttg	ttt	aaa	gta
K	E	Q	L	K	Q	L	E	A	L	F	K	V
								300				
agt	tgg	gag	cag	ctc	tct	gcg	ggt	gat	ttt	act	ttt	gct
S	W	E	Q	L	S	A	G	D	F	T	F	A
												350
ctg	ctg	tta	cct	gat	ggt	aat	gct	gcg	tta	act	gag	cgt
L	L	L	P	D	G	N	A	A	L	T	E	R
gcg	agt	tta	tta	tgt	gcc	tgg	act	caa	ggc	ttt	tta	act
A	S	L	L	C	A	W	T	Q	G	F	L	T
			400									
ggc	ttg	cat	tta	tc								
G	L	H	L									

Figure 1 – SEQ 1 - Partial nucleotide sequence of Psclone 51A and the deduced protein

```
1     atgagtcaag cattagttga atttaaaacg gtagatcatg tgctgcaagc
51    cttaggcgtt gaagggatga gtgcagcgga tgtgcatggc ctgctgattg
101   gtatgttggc gagtcaaagt aatttaacct gtaaatcatg gttagaaaaa
151   gcgatattta tgggagctca ccttgatgct gaaagtgatc tttttagtaa
201   tatcatggcc aaagagcagt taaagcagtt agaggcattg tttaaagtaa
251   gttgggagca gctctctgcg ggtgatttta cttttgctct gctgttacct
301   gatggtaatg ctgcgttaac tgagcgtgcg agtttattat gtgcctggac
351   tcaaggcttt ttaactggct tgcatttatc gggtgttaat attgctaaat
401   ataaagaagg tgaattagcg acgaccttaa aagatttagc tgaagttgcg
451   cagttggatt tagccattga agacagtaat gaaaatgaag cggcatatac
501   tgagattgct gaatatgtac gtatggcggc gctttttgtt catagtgaat
551   tagccggttc tggtcaagcg actcagatga ctgttcatta a
```

Figure 2 – SEQ ID 2 – Complete coding sequence of Psclone51A

```
1     MSQALVEFKT VDHVLQALGV EGMSAADVHG LLIGMLASQS NLTCKSWLEK
51    AIFMGAHLDA ESDLFSNIMA KEQLKQLEAL FKVSWEQLSA GDFTFALLLP
101   DGNAALTERA SLLCAWTQGF LTGLHLSGVN IAKYKEGELA TTLKDLAEVA
151   QLDLAIEDSN ENEAAYTEIA EYVRMAALFV HSELAGSGQA TQMTVH*
```

Figure 3 – SEQ ID 3 - Protein sequence derived from the ORF of the Psclone51A

```
atgaacagaggatgtttgcaaggtagtagtctaattattatcagtgtgtt
tttagttggctgtgcccagaactttagtcgtcaagaagtcggagctgcga
ctggggctgttgttggcggtgttgctggccagctgtttggtaaaggtagt
ggtcgagttgcaatggccattggtggtgctgttttgggtggattaattgg
ttctaaaatcggtcaatcgatggatcagcaggataaaataaagctaaacc
agagtttggaaaaggtaaaagcagggcaagtgacacgttggcgtaatcca
gatacaggcaatagttatagtgttgagccagtgcgtacttaccagcgtta
caataagcaagagcgtcgccagcaatattgtcgagaatttcagcaaaagg
cgatgattgcagggcagaagcaagagatttacggcactgcatgccggcaa
ccggatggtcgttggcaagtcatttcaacagaaaaataa
```

Figure 4 – SEQ ID 4 – Nucleotide sequence of P10.6

```
MNRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGS
GRVAMAIGGAVLGGLIGSKIGQSMDQQDKIKLNQSLEKVKAGQVTRWRNP
DTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQEIYGTACRQ
PDGRWQVISTEK
```

Figure 5 – SEQ ID 5 – Amino Acid Sequence of 10.6

```
1
caa gga ata ttg gca aaa tgg gag aat gtt tct gct caa aaa gtg atg gtt
 Q   G   I   L   A   K   W   E   N   V   S   A   Q   K   V   M   V
52
gcc aag gtt agt aca gtt aat aat gct gga gat aat ggt cag tca ggt aaa
 A   K   V   S   T   V   N   N   A   G   D   N   G   Q   S   G   K
103
aat aat aat gaa aat att att gaa aaa gca ggt gct att gta ttt gcg gta
 N   N   N   E   N   I   I   E   K   A   G   A   I   V   F   A   V

                                        XmaI
                                        SmaI
154                                     ----------
ctc gat aca cag cta aac agt gac caa ccc ggg act cca gta atg gca acg
 L   D   T   Q   L   N   S   D   Q   P   G   T   P   V   M   A   T
205
att gtt caa ggt aaa ttt aaa aat gcc aaa ttg ttg ggt agc ttt aaa aga
 I   V   Q   G   K   F   K   N   A   K   L   L   G   S   F   K   R
256
gag gat gaa aaa cta gtc att tct ttt gat cgc ata tct ttg cct gaa ctt
 E   D   E   K   L   V   I   S   F   D   R   I   S   L   P   E   L
307
gat cac agt att tct att aag gcg tat gca att aat gcc aca aca gca caa
 D   H   S   I   S   I   K   A   Y   A   I   N   A   T   T   A   Q
358
aat gca ctg tct tca gat gta gat aat cat tat tta tta cgt tat ggt ggg
 N   A   L   S   S   D   V   D   N   H   Y   L   L   R   Y   G   G
409
ctc ttt gct gct gcg ttt ttg caa ggc ttt ggc gat tat ttc tcc caa aac
 L   F   A   A   A   F   L   Q   G   F   G   D   Y   F   S   Q   N

      Hind III
460   ----------
tca tca agc tta tgt ggt ggt gcg aca acc tgt att att aca ggc act caa
 S   S   S   L   C   G   G   A   T   T   C   I   I   T   G   T   Q

        PstI
511   ----------
tca act gca gaa caa aat cgt aca acg aga aaa gcc cta tat tct ggt tta
 S   T   A   E   Q   N   R   T   T   R   K   A   L   Y   S   G   L
562
ggt caa gtt gga aca act tta gct ggt aaa gca agc gct gca ttt gat cgc
 G   Q   V   G   T   T   L   A   G   K   A   S   A   A   F   D   R
```

Figure 6 - SEQ ID 6 - DNA sequence of P. salmonis IcmE and encoded protein (5' to 3')

```
613
cct cca acg gtt act tta aat caa ggt gtt ggt atg ggg att tta ttt atg
 P   P   T   V   T   L   N   Q   G   V   G   M   G   I   L   F   M
664
tcg gat gta aag gtg taa gtt aaa atg agt aat aat cac aca gat aaa aat
 S   D   V   K   V
715
tat aat ttt gat gat gat caa gac att aat gag gat aaa gaa gac ctt gcn

766
gct aga taa tga gcc agc agt taa aag aaa taa tgc aga tgc tgt ttg gca

817
agg gac ttc act gtg gga taa aat aag gcc gat gtt gca tta tta tat cat

868
tgc tat tat tgc att cgc tgt agc agg tta tat gat gta taa cgc ata ccg

919
aac ttt ata tcc aaa gca gtc agt gca gca ggc tga ggc taa cca ttt aag

970
ctt tag taa tca ggt tga aac tgg cag taa gtc agc gaa agg ctt ttc tcc

1021
cct ggc tca gtc tca aga aaa taa ggt caa aaa taa aag tgg gnc ttg gaa

1072
aaa aag aag aga taa aac cga atg cta
```

Figure 6 –(continued) - SEQ ID 6 DNA sequence of P. salmonis IcmE and encoded protein (5' to 3')

ADNGKLQLQI SQLKAQQTQL QQQVANLQGQ

Figure 7 – SEQ ID 7 – Amino acid sequence of P. salmonis p45 antigen (N-terminal to C-terminal)

```
ga      tct     tta     atc     tca     tta     aaa     ttt     atg     ttt     cta     atc     gcg
                                                                                    D       R
tgg     gtt     att     ttc     atc     aaa     tac     ggc     att     ttt     agg     agg     agg
P       N       N       E       D       F       V       A       N       K       P       P       P
gat     aac     aac     atc     agc     att     agg     tga     gtg     gtt     taa     aac     gga
I       V       V       D       A       N       P       S       H       N       L       V       S
                                                                            PsII
atc     gta     aac     atc     atg     gct     atc     gta     ggc     tcc     gtc     tgc     agt
D       Y       V       D       H       S       D       Y       A       G       D       A       T
gaa     gcg     atc
F       R       D
```

Figure 8 – SEQ ID 8- DNA sequence of P. salmonis clone 3/original and encoded protein (3'to 5')

```
ata     agc     cta     gat     gat     gat     att     gcg     gga     ata     ncc     att     gat
I       S       L       D       D       D       I       A       G       I       X       I       D
tna     cag     gcc     tta     agc     gtt     ttg     gcc     gtg     acg     atg     tgg     cac
X       Q       A       L       S       V       L       A       V       T       M       W       H
caa     gaa     aaa     tac     aag     ata     tca     gca     aag     cgc     agc     tgg     cgt
Q       E       K       Y       K       I       S       A       K       R       S       W       R
aaa     ctt     cat     gtg     gcc     gtt     gat     gat     gat     can     tat     att     caa
K       L       H       V       A       V       D       D       D       X       Y       I       Q
gcc     gca     ctc     atc     acc     gat     cgc     tat     gaa     gca     gat     gag     gag
A       A       L       I       T       D       R       Y       E       A       D       E       E
```

Figure 9 – SEQ ID 9 – DNA sequence of P. salmonis clone 3/3PST-R and encoded protein (5'to 3')

```
tat     gag     att     aaa     gat     cat     ggt     aga     atg     cac     tgg     caa     aag
Y       E       I       K       D       H       G       R       M       H       W       Q       K
aca     cga     caa     tac     ggc     aag     cgt     aat     tat     tct     gag     ttg     gcg
T       R       Q       Y       G       K       R       N       Y       S       E       L       A
att     cag     cgt     tac     aaa     cgc     att     ttg     ggc     aac     acg     atg     cag
I       Q       R       Y       K       R       I       L       G       N       T       M       Q
tcc     aga     gac     ata     tcg     cga     cag     aaa     aat     gaa     gga     cta     att
S       R       D       I       S       R       Q       K       N       E       G       L       I
ggc     gcg     ggt     att     tta     aat     aga     gat     gac     can     tct     cgg     cat
G       A       G       I       L       N       R       D       D       X       S       R       H
gcc     ggt     gac     aat     aat     gta
A       G       D       N       N       V
```

Figure 10 – SEQ ID 10 – DNA sequence of P. Salmonis clone 3/3APA-F and encoded protein (5' to 3')

g	atc	aan	gcc	cgc	ata	tta	atc	gac	gac	cac	gat	att
	I	X	A	R	I	L	I	D	D	H	D	I
caa	aag	tta	aaa	att	caa	aat	atc	cgc	caa	cat	att	gcc
Q	K	L	K	I	Q	N	I	R	Q	H	I	A
tat	tta	cct	cag	cat	ggt	gac	tta	ttt	aat	ggc	acg	atc
Y	L	P	Q	H	G	D	L	F	N	G	T	I

Figure 11 - SEQ ID 11 – DNA sequence of P. salmonis clone 7/orginal and encoded protein (5' to 3')

g	cac	gca	tat	cga	tac	cta	acg	tcn	ttt	caa	gaa	caa
	H	A	Y	R	Y	L	T	S	F	Q	E	Q
aaa	tat	tat	aaa	caa	gcc	ata	gaa	gtc	agc	caa	tta	ctt
K	Y	Y	K	Q	A	I	E	V	S	Q	L	L
ggc	ctt	gac	tca	att	att	gag	cgc	ttg	ccc	aaa	ggc	tat
G	L	D	S	I	I	E	R	L	P	K	G	Y
cac	act	cct	gtt	gcc	aat	cat	gcc	gna	tat	taa	ttg	act
H	T	P	V	A	N	H	A	X	Y			

Hind III

acg	cac	gat	ntt	nca	aag	ctt	ant	g

Figure 12 - SEQ ID 12 – DNA sequence of P. salmonis clone 7/Xbar and encoded protein (5' to 3')

tta	ttg	agc	gct	tgc	cca	aag	gct	atc	aca	ctc	ctg	ttg
L	L	S	A	C	P	K	A	I	T	L	L	L
cca	atc	atg	cca	tgg	nag	tcg	cta	cct	cgc	ggt	atc	att
P	I	M	P	W	X	S	L	P	R	G	I	I
cag	cgc	att	gcg	att	gcc	cgt	gcc	ctg	att	cat	aag	cca
Q	R	I	A	I	A	R	A	L	I	H	K	P
cca	atc	gtc	cta	ttc	gat	gag	gcc	aat	acg	gcc	atg	gac
P	I	V	L	F	D	E	A	N	T	A	M	D
atg	caa	ggt	gat	acc	atc	tta	att	aat	gtg	ctt	gaa	caa
M	Q	G	D	T	I	L	I	N	V	L	E	Q
ctt	aaa	ggc	acc	tgc	aca	ctc	atc	ctc	gtc	tct	cat	cgc
L	K	G	T	C	T	L	I	L	V	S	H	R

XhoI

PaeR71

cca	tca	ttg	ctg	gca	cat	gca	gat	aaa	atc	ttt	atc	ctc
P	S	L	L	A	H	A	D	K	I	F	I	L

gag	aat	aaa	aat	ctg	gtg	gag	aaa	gtc	aca	tga	gct	ctg
E	N	K	N	L	V	E	K	V	T			
cac	taac	cgc	cca	gga	gca	taa	tat	tcg	cac	tgc	gtt	tat
taa	cag	cct	cga	acc	act	gtt	aac	tgc	att	agg	ctg	gc

Figure 13 – SEQ ID 13 – DNA sequence of P. salmonis clone 7/MunR and encoded protein (5' to 3')

ggt	tgt	tgn	tgg	aat	atc	aag	gct	aat	att	ggt	aaa	tng
		X	P	I	D	L	S	I	N	T	F	X
cca	att	ttc	atc	atc	ttc	att	tgt	gct	tgt	att	att	att
W	N	E	D	D	E	N	T	S	T	N	N	N
ttt	atc	ata	acg	aaa	att	gac	atg	ttc	aaa	gca	aat	gtg
K	D	Y	R	F	N	V	H	E	F	C	I	H
gcc	atc	aat	atg	ggc	gag	ttg	ctt	ttg	cgt	ggg	aac	ata
G	D	I	H	A	L	Q	K	Q	T	P	V	Y
ctc	tgg	agg	gag	ttt	aaa	gac	ttc	ttg	tac	att	tat	ctt
E	P	P	L	K	F	V	E	Q	V	N	I	K
cag	cac	tac	caa	tcg	act	gta	agc	ggt	tcc	aga	cgg	agg
L	V	V	L	R	S	Y	A	T	Q	S	P	P
tng	cac	gat	taa	ttg	gct	gtn	nac	agc	gcc	ctg	aca	gtn
X	V	I	L	Q	S	X	V	A	G	Q	C	X
acg	gtg	c										
R	H											

Figure 14 – SEQ ID 14 – DNA sequence of P. Salmonis clone 7/MunF and encoded protein (3'to 5')

gg	atc	atg	ctc	atc	ctc	tac	gtc	gat	gcg	atg	gtc	tca
	I	M	L	I	L	Y	V	D	A	M	V	S
cct	tta	gga	acg	gct	tta	gcc	tat	acc	ggc	tct	tct	aca
P	L	G	T	A	L	A	Y	T	G	S	S	T
cgg	atg	cta	acg	gcc	atg	tct	cgc	gaa	aaa	cag	gtt	ccg
R	M	L	T	A	M	S	R	E	K	Q	V	P
cgt	ttc	ttt	gac	cat	gta	cac	ccc	cac	tat	ggt	gtt	tcc
R	F	F	D	H	V	H	P	H	Y	G	V	S
cgt	cgt	tca	ttg	atc								
R	R	S	L	I								

Figure 15 – SEQ ID 15 – DNA sequence of P. Salmonis clone 20/original and encoded protein (5' to 3')

g	atg	agc	atg	atc	ata	tgc	agg	cct	aat	aga	ccg	gct
aac	tgt	acc	atg	ggt	gct	tga	aag	tca	agc	tgg	tgc	cag
cca	ttt	tta	agc	ata	ctt	ggc	ggt	agt	gca	cca	ata	aat
			A	Y	K	A	T	T	C	W	Y	I
gac	act	tga	caa	gag	caa	gta	aat	cac	taa	gca	gat	tac
V	S	S	L	L	L	Y	I	V	L	C	I	V
aat	cga	gag	gac	cag	aga	gag	cgg	gat	att	gcg	ttt	tgg
I	S	L	V	L	S	L	P	I	N	R	K	P
gtt	tga											
N	S											

Figure 16 – SEQ ID 16 DNA sequence of P. salmonis clone 20/20VSPF and encoded protein (3' to 5')

ClaI

ggt	aaa	nga	gta	tcg	ata	ttg	gcg	ttt	ttt	ggc	tgt	att
G	K	X	V	S	I	L	A	F	F	G	C	I
tta	tgg	ttc	agg	ttg	tgc	gag	tac	ggt	gcc	aac	agg	gcg
L	W	F	R	L	C	E	Y	G	A	N	R	A
cct	tat	tct	gat	tat	cct	cat	gtg	tat	gca	tgc	ccg	aat
P	Y	S	D	Y	P	H	V	Y	A	C	P	N
aag	tta	agt	act	ttg	tgt	tat	cgt	aca	gcg	att	gca	ccg
K	L	S	T	L	C	Y	R	T	A	I	A	P
gtt	gga	cac	tgg	tct	cag	tat	aat	cag	ctg	agc	ttt	cag
V	G	H	W	S	Q	Y	N	Q	L	S	F	Q
ttg	ccg	att	gct	ttg	caa	gta	cca	ttg	cgt	caa	gga	caa
L	P	I	A	L	Q	V	P	L	R	Q	G	Q
tta	gag	cta	caa	gag	tat	tat	gct	aaa	aat	ccc	gta	ttg
L	E	L	Q	E	Y	Y	A	K	N	P	V	L
cct	tca	tct	ttg	cct	tta	tca	ggc	cca	ggc	ccg	tta	acg
P	S	S	L	P	L	S	G	P	G	P	L	T
tct	tat	tta	tat	cca	ttt	gga	ttg	tgt	gca	aca	aaa	ata
S	Y	L	Y	P	F	G	L	C	A	T	K	I
att	cgc	tta	gag	agt	tta	act	gat					
I	R	L	E	S	L	T	D					

Figure 17 – SEQ ID 17 – DNA sequence of P. Salmonis clone 15/original and encoded protein (5' to 3')

MAEIIGIDLG	TTNSCVAVLD	GDKPRVIESA	EGDRTTPSIV
AYTNDGVTVG	QPAKRQAVTN	PNNTLFAVKR	LIGRKSSDDT
VQRDIERLPY	TIAAADNGDA	WIDVNGEKLA	PPQISAQVLA
KMKKTAEDYL	GEDVKEAVIT	VPAYFNDAQR	QATKDAGRIA
GLDVKRIINE	PTAAALAYGM	DKKRGDGVIA	VYDLGGGTFD
ISIIEIAEVD	GEHQFEVLAT	NGDHTLGGED	FDLRLISYLV
	Missing amino acids		
DEFKKEQGID	XXXXXXXXXX	XXEASEKAKI	ELSSTQQTDV
NLPYITADAT	GPKHMNIRVT	RAKFESLVED	LVEGTIEPCR
VALKDAGLSV	NDVTDVILVG	GQTRMPKAQA	VVKNFFGKEP
RRDVNPDEAV	AVGAAIQGGV	LAGDVKDVML	LDVTPLSLGI
ETMGGVMTKL	IEKNTTIPTK	SQTFSTAQDN	QNAVTVHVLQ
GEREVATGKK	LTGRFDLADI	PPAPRGMPLI	LRVHFDIDAN
GTLNVSAKDK	GTGKEQSIVI	RRSSGLSDDE	VDAMIKDAED
HADDDKKFQE	LVGARNNAEA	MIHATEKGLK	EADGKVAADD
KTAIEKAISE	LKDVVSGLDK	AVIDEKVEAL	TQASAKMAEV
LYANQGAEAE	AAAAGAEQAQ	SQTDEKKDDD	VVDAEFEEV

Figure 18 – SEQ ID 18 – Amino acid sequence of P. Salmonis HSP70 protein (N-terminal to C-terminal)

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WO 01/68865 A3

(54) Title: FISH VACCINE AGAINST PISCIRICKETTSIA SALMONIS

(57) Abstract: The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against infection by *Piscirickettsia salmonis*. The invention is based on or derived from the nucleic acid or amino acid sequence of at least a part of the surface antigen present on *Piscirickettsia salmonis*. Nucleic acid and/or amino acid sequences may be used in the preparation of a vaccine to protect against infection by *Piscirickettsia salmonis*.

INTERNATIONAL SEARCH REPORT

Int ational Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/29 A61K39/02 A61K48/00 A61P31/04

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B. FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, CHEM ABS Data, BIOSIS, SEQUENCE SEARCH, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JONES S R ET AL: "Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon Salmo salar in eastern Canada." DISEASES OF AQUATIC ORGANISMS, (1998 MAY 14) 33 (1) 25-31. , XP001029879 the whole document ---	3-8
A	BARNES M N ET AL: "Purification of Piscirickettsia salmonis and partial characterization of antigens." DISEASES OF AQUATIC ORGANISMS, (1998 MAY 14) 33 (1) 33-41. , XP001029898 the whole document ---	3-8

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Mennessier, T

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARSHALL S ET AL: "Minimally invasive detection of Piscirickettsia salmonis in cultivated salmonids via the PCR." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1998 AUG) 64 (8) 3066-9. , XP001029515 the whole document	3-8
P,X	KUZYK M A ET AL: "OspA, a lipoprotein antigen of the obligate intracellular bacterial pathogen Piscirickettsia salmonis." JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, (2001 JAN) 3 (1) 83-93. , XP001029507 the whole document	3-8

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-2 (each as a whole), 3-5 (each partly)

Due to the indefinite expressions "substantially homologous to at least a part of the surface antigen" used in claim 1 and "a part of the surface antigen" used in claim 2, no meaningful search could be carried out on the subject-matter of said claims.

Whereas the same defect also affects claims 3-5 as being dependent on said claims, a meaningful partial search could be carried out on said claims taking into consideration only the whole nucleic acid and amino acid sequences referred to in claims 3 and 5, i.e., those sequences having the sequence identifiers SEQ ID NO: 1-8 and 10-29, as defined in the listing sequence filed with the letter of 25.06.2001. The whole sequences were searched as well as the use of the whole nucleic acid sequences in the preparation of a vaccine.

With respect to claim 6-8, a complete search was carried out, the SEQ ID of the sequence listing filed with the letter of 25.06.2001 being taken into consideration (SEQ ID NO: 1, 3, 5, 7, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 for the nucleic acid sequences; SEQ ID NO: 2, 4, 6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 30 for the amino acid sequences).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.